

In the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously Presented) A recombinant herpes simplex virus, comprising a heterologous nucleotide sequence selected from the group consisting of the sequences represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5.

2. (Previously Presented) The recombinant herpes simplex virus according to claim 1, wherein the heterologous nucleotide sequence is inserted at the XbaI site of the UL2 gene or the UL44 gene of the HSV genome.

3. (Previously Presented) The recombinant herpes simplex virus according to claim 1, wherein the heterologous sequence represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5 is inserted in a nonessential gene region of the HSV genome.

4. (Previously Presented) A method for the production of a recombinant herpes simplex virus, comprising obtaining at least one DNA segment represented by a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5; and inserting the DNA segment into an HSV genome using gene engineering method(s), thereby obtaining the recombinant herpes simplex virus.

5. (Canceled)

6. (Previously Presented) A method for large-scale production and preparation of a recombinant adeno-associated virus serotype 1, 3, 4, 5, or 6, the method comprising:

(1) obtaining a recombinant herpes simplex virus comprising a heterologous nucleotide sequence selected from the group consisting of the sequences represented by SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5;

(2) establishing “a vector cell”, namely, a recombinant AAV vector cell strain;

- (3) infecting the vector cell strain with the recombinant herpes simplex virus; and
- (4) culturing the infected vector cell strain under conditions whereby recombinant adeno-associated viruses are produced.

7. (Currently Amended) A method for isolation and purification of recombinant adeno-associated virus (rAAV) comprising:

1)obtaining a crude lysis solution comprising rAAV-containing cells, wherein the rAAV has a serotype selected from the group consisting of serotypes 1, 3, 4, 5, and 6; and  
wherein the rAAV has been produced as a result of use of the recombinant herpes simplex virus of claim 1;

2)adding chloroform to ~~at~~the crude lysis solution comprising recombinant adeno-associated virus-containing cells to deactivate any HSV helper viruses, lyse cells, and denature and precipitate a great many cell proteins to obtain cell lysis solution;

2)3)adding solid sodium chloride to the cell lysis solution until the final concentration is 1.0 to 1.2 mol/L with stirring for dissolution, then centrifugating the mixture and leaving the supernatant;

3)4)precipitating rAAV with PEG/NaCl, adding solid polyethylene glycol to the sodium chloride-containing supernatant of step 2) with stirring for dissolution, letting the mixture sit, then centrifugating the mixture and discarding the supernatant but leaving the precipitate;

4)5)treating the cell lysis solution with DNaseI and RNase to degrade the nucleic acid, dissolving the precipitate of step 3), and adding DNaseI and RNase to dissolve the residual nucleic acid apart from the rAAV viral particles;

5)6)using chloroform to extract and remove other proteins and the residual PEG, adding chloroform to extract, and then, centrifugating the mixture and removing the upper water phase;

6)7)removing salts via dialysis; and

7)8)further purifying rAAV via density gradient centrifugation or affinity chromatography.

8. (Currently Amended) A recombinant vector plasmid pSNAV-NX, said plasmid comprising an ITR at each of the two ends of an AAV genome selected from the group consisting of the AAV-1, AAV-3, AAV-4, AAV-5 and AAV-6 genomes; wherein said AAV genome is a recombinant AAV genome produced with the recombinant herpes simplex virus of claim 1; and further comprising an immediate early enhancer and a promoter of cytomegalovirus, a multiple cloning site and a polyA signal successively intervening between the two ITRs, and a neomycin resistant gene expression cassette flanking the outside of at least one of the ITRs.

9. (Canceled)

10. (Currently Amended) A method for purification of recombinant adeno-associated virus serotype 1, 3, 4, 5, or 6, the method comprising: obtaining a solution containing rAAV of serotype 1, 3, 4, 5, or 6; said rAAV having been produced with the herpes simplex virus of claim 1; adjusting the conductance value of obtained rAAV solution before passing through an ion exchange column which has been balanced by a buffer, balancing the ion exchange column using a buffer again; eluting the ion exchange column with a salt-containing buffer and collecting the elution peaks; passing the collected elution peaks through a molecular sieve column which has been balanced by a buffer; and washing the column with a buffer again.

11. (Previously Presented) The method of claim 7 wherein the rAAV is an empty capsid of an AAV serotype selected from the group consisting of serotypes 1, 3, 4, 5, and 6.